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Neuren Pharmaceuticals Ltd
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Auckland 1001

Patent Application Number: 520866
In the Name of: AUCKLAND UNISERVICES LIMITED
Patent Title: Modulation of density, distribution and signal transduction potential of angiotensin II receptors
Your Reference: B1006L

Certificate of Commissioner

Thank you for your request dated 26 July 2006.

Attached is a copy of the certificate.

Please contact me if you require further information.

Yours sincerely

D Seiringer

Daisy Seiringer
Business Support Officer
For Commissioner of Patents, Trade Marks and Designs
Direct Dial (04) 9783654
mail@iponz.govt.nz

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CERTIFICATE

I hereby certify that this is a true copy of the application as filed on 19 August 2002 with an application for Letters Patent number 520866 made by AUCKLAND UNISERVICES LIMITED. I further certify that this application was voided pre-acceptance on the 19 November 2003.

Dated 28 July 2006.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

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330 High Street, Lower Hutt
PO Box 30687, Lower Hutt, New Zealand or DX SX 11129, Wellington
Freephone: 0508 4 IPONZ (0508 447 669), International callers dial: +64 3 962 2607, Fax: +64 4 560 1691, www.iponz.govt.nz

520666

Our Ref:

Patents Form No 1.

New Zealand Patents Act 1953
Application for a Patent

We **AUCKLAND UNISERVICES LIMITED** of UniServices House, Level 10, 70 Symonds Street, Auckland, are in possession of an invention which is described in the accompanying provisional specification under the title

MODULATION OF DENSITY, DISTRIBUTION AND SIGNAL TRANSDUCTION POTENTIAL OF ANGIOTENSIN II RECEPTORS

We believe **MARK HEDLEY VICKERS**, a British citizen of 22 Kerswill Place, Pakuranga, Auckland, New Zealand and **BERNHARD HERMANN HEINRICH BREIER**, a German citizen of 1 Edenvale Crescent, Mount Eden, Auckland, New Zealand, to be the true and first inventors of the invention, and we are the assignee of the said inventors in respect of the right to make this application.

We declare that to the best of our knowledge and belief the statements made above are correct and there is no lawful ground of objection to the grant of a patent to us on this application, and we pray that a patent may be granted to us for the said invention.

And we request that all notices, requisitions, and communications relating to this application may be sent to

Buddle Findlay
PricewaterhouseCoopers Tower
188 Quay Street
PO Box 1433
Auckland 1001
New Zealand

Who are hereby appointed by us to act for us in relation to this application.

Dated the 16th August 2002.

AUCKLAND UNISERVICES LIMITED

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PROVISIONAL SPECIFICATION

MODULATION OF DENSITY, DISTRIBUTION AND SIGNAL TRANSDUCTION POTENTIAL OF ANGIOTENSIN II RECEPTORS

We, **UNISERVICES LIMITED** of UniServices House, Level 10, 70 Symonds Street, Auckland do hereby declare this invention to be described in the following statement.

MODULATION OF DENSITY, DISTRIBUTION AND SIGNAL TRANSDUCTION POTENTIAL OF ANGIOTENSIN II RECEPTORS

FIELD OF INVENTION

This invention relates to the field of therapeutic and/or prophylactic use of insulin-like growth factor-I to modulate the density, distribution and the potential for signal transduction of angiotensin II receptors or angiotensin II-like G protein-coupled seven transmembrane receptors in mammalian tissue.

In particular, the invention relates to the method for modulating the density, distribution, and the potential for signal transduction of angiotensin II receptors in mammalian renal tissue by administering insulin-like growth factor–I compound to a mammal.

BACKGROUND ART

Ang II and its receptors have been implicated in the development of various diseases. These ANG II-mediated diseases include hypertension, cardiac insufficiency, ischemic peripheral circulation disturbances, myocardial ischemia, vein insufficiency, progressive cardiac insufficiency after myocardial infraction, diabetic nephritides, nephritis, arteriosclerosis, hyperaldosteronism, dermatosclerosis, glomerulosclerosis, renal insufficiency, diseases of central nervous system, sensory disturbances including Alzheimer's disease, deficiency of memory, depression, amnesia, senile dementia, anxiety neurosis, catatonia or indisposition, glaucoma, and intraocular hypertension. It is well accepted that AT_1 receptor stimulation contributes to development of hypertension (Sandberg et al. 2000) and that AT_1 blockade in patients with hypertension not only reduces blood pressure, but also improves arterial compliance (Siragy et al. 2001; Kelmsdal T et al. 1999).

A group of antihypertensive drugs, ANG II antagonists, has been developed which comprises substances that bind to, but do not result in the activation of AT_1 receptors (AT_1R). Presently ANG II antagonists like candesartan, irbesartan, losartan, telmisartan and valsartan are selective for AT_1R. It has been proven in the literature that inhibition of the rennin-angiotensin system (RAS) by angiotensin-converting enzyme (ACE) inhibition or blockade of AT_1Rs has a positive influence not only on blood pressure (BP), but also achieves BP independent renoprotective effects. (Adamczak et al. 2002). The selective

ANG II receptor antagonism has been show to reduce insulin resistance and improve glucose tolerance. (Henriksen et al. 2001).

The applicants' finding points to insulin-like growth factor-I (IGF-I) as a new alternative therapy or a co-therapy in a number of ANG II-mediated conditions, in particular hypertension or hypertension related renal diseases.

Present invention discloses that administration of IGF-I results in the modulation of density, distribution and signal transduction of angiotesin II receptors in mammalian kidney. The applicants have previously observed that administration of IGF-I reduces insulin resistance and improves glucose tolerance (Vickers et al. 2001), and thus, IGF-I administration achieves the beneficial effects of selective angiotensin II receptor antagonism comparable to those of angiotensin antagonists and ACE inhibitors. Moreover, the method disclosed in the present application has beneficial side effects not achieved by standard anti-hypertensive drugs. For example, modulation of age and fat mass regulated adipocyte angiotensin II receptors by IGF-I can prevent adipose tissue hypertrophy and ameliorate obesity.

The novel application of IGF-I disclosed in the present invention provides the public with a beneficial alternative to the methods of blocking or inhibiting the action of RAS known in the prior art. Moreover, the present invention describes a new method of enhancing the efficacy of the known methods of inhibiting ANG II activity.

OBJECT OF THE INVENTION

Accordingly, it is the object of this invention to provide a method of modulating the density, distribution and the potential for signal transduction of ANG II receptors or ANG II-like G-protein-coupled seven transmembrane receptors expressed in mammalian tissue. The method provided in the invention may be used as an independent treatment or as a co-treatment in a number of ANG II-mediated conditions. In particular, though not exclusively, the method will be beneficial in treatment of hypertension, hypertension related renal diseases or obesity.

SUMMARY OF THE INVENTION

Accordingly, in a broad aspect the present invention comprises a method for modulating the density, distribution and the signal transduction potential of ANG II receptors or ANG II-like G protein-coupled seven transmembrane receptors in mammalian tissue comprising the step of administering an effective amount of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs to a mammal.

Preferably the ANG II-like G protein-coupled seven transmembrane receptors are G protein-coupled seven transmembrane receptors having a pressor or depressor activity.

Preferably the ANG II receptors are ANG II type 1 receptors and their density, distribution and signal transduction potential is down-regulated.

Preferably the ANG II receptors are ANG II type 2 receptors and their density, distribution and signal transduction potential are up-regulated.

Preferably the mammal is human.

Preferably the angiontensin II receptors or angiotensin II-like G protein-coupled seven transmembrane receptors are located in mammalian renal tissue, most preferably in glomeruli; glomerular mesangial cells; inner stripe of the outer medulla; outer stripe of the outer medulla; inner medulla toward the tip of the papilla; proximal convoluted tubules; proximal tubular epithelia; vascular smooth muscle cells, in particular, efferent arteriolar vascular smooth muscle cells; on luminal surface of proximal and distal tubule cells.

Preferably the effective amount of an insulin-like growth factor-I (IGF-I) compound is administered in a form of a pharmaceutical composition including a pharmaceutically acceptable carrier thereof.

Preferably the effective amount of IGF-I compound is administered by way of administration of a replicable vehicle encoding for said IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs.

Preferably the effective amount of IGF-I compound is administered by intramuscular, subcutaneous or intraperintoneal injection or implant.

Preferably the said effective amount of IGF-I compound is administered through intravenous, transdermal, transmucosal, oral, or epidural route.

Preferably the effective amount of an insulin-like growth factor-I (IGF-I) compound is between 0.01mg/kg/day and about 1mg/kg/day.

In a further aspect the present invention provides for a method for modulating the density, distribution and the potential for signal transduction of G protein-coupled seven transmembrane receptors in a mammalian tissue comprising the step of administering an effective amount of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs to a mammal.

In a further aspect the present invention provides for the use of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs for the manufacture of a medicament for modulation of the density, distribution and the potential for signal transduction of angiotensin II receptors or angiotensin II-like G protein-coupled seven transmembrane receptors in a mammalian tissue.

Preferably the medicament is administered in a pharmaceutically acceptable combination with one or more suitable carriers or excipients.

Preferably medicament is to be used for treatment, prophylaxis, attenuation of hypertension in the mammal.

Preferably the medicament is to be used for treatment, prophylaxis, attenuation of resulting from hypertension related kidney diseases in a mammal.

Preferably the medicament will be administered in the presence of ACE inhibitors or angiotensin II antagonists.

Preferably the ACE inhibitor is selected from a group that includes but is not limited to captopril, cilazapril, enalapril, fosinopril, imidapril, lisinopril, moexipril, perindopril, quinapril, ramipril, or trandolapril.

Preferably the angiotensin II antagonist is selected from a group that includes but is not limited to candesartan, irbesartan, losartan, telmisartan or valsartan.

In a further aspect the present invention provides for the use of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs for the manufacture of a medicament for modulation of the density, distribution or the potential for signal transduction of G protein-coupled seven transmembrane receptors in a mammalian tissue.

In a further aspect the present invention comprises a method for enhancing the antihypertensive and renoprotective properties of ACE inhibitors and ANG II antagonists comprising the step of administering to a mammal an effective amount of an IGF-I compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs in the presence of the said ACE inhibitor or the said angiotensin II antagonist.

BRIEF DESCRIPTION OF FIGURES

The invention will be better understood by way of example with reference to the accompanying figures.

FIG. 1 Shows the photomicrograph of an immunohistochemical section of a programmed kidney incubated with the AT_1R antibody. Localisation of the AT_1R immunoreactivity (brown staining) can be seen distinctly in the medullary region (MR). Slight immunoreactivity is also evident in the cortical region (CTX). (Mag 100x).

FIG. 2 Shows the photomicrograph of the negative control immunohistochemical kidney incubated with normal rabbit serum. No evidence of AT_1R immunoreactivity was observed. (Mag x 50).

FIG. 3 Shows the photomicrographs of an immunohistochemical section of a programmed kidney incubated with the AT_1R. Renal cortex demonstrates labelling throughout the glomeruli (Glm) and renal tubules, specifically the proximal (PT) and distal (DT) tubules. (A: mag 250x, B: mag 1000x).

FIG. 4 Shows the photomicrographs of an immunohistochemical section of a programmed kidney treated with IGF-1 incubated with the AT_1R. There is no evident labelling throughout glomeruli and renal tubules. (A: mag 250x, B: mag 1000x).

FIG. 5 Shows the photomicrograph of an outer medullary immunohistochemical section of a programmed kidney incubated with the AT_1R. Distinct labelling can be seen in the renal tubules. (mag 250x)

FIG. 6 Shows the photomicrograph of the outer medullary immunohistochemical section of a programmed kidney treated with IGF-1 and incubated with the AT_1R. Decreased AT1R immunoreactivity is seen (mag 250x).

FIG. 7 Shows the photomicrograph of the outer medullary immunohistochemical section of a programmed kidney incubated with the AT_1R. Strong labelling of the proximal tubules is demonstrated with lesser staining within the distal tubules (mag 630x).

FIG. 8 Shows the photomicrograph of the outer medullary immunohistochemical section of a programmed kidney treated with IGF-1, incubated with the AT_1R. Little immunoreactivity is seen with both the proximal (T) and distal (DT) tubules (mag 630x).

FIG. 9 Consists of the pistogram showing the localisation and intensity of the AT_1R in the programmed offspring.

Values are expressed as mean ± SEM.

DETAILED DESCRIPTION OF THE INVENTION

Angiotensin II (ANG II) and angiotensin II binding receptors play a key role in the renin-angiotensin system (RAS) which is responsible for hormonal control of blood pressure and sodium and water homeostasis. Renin, produced mainly in the renal juxtaglomerular apparatus, acts on angiotensinogen, present in the blood, kidney and other organs, to produce angiotensin I (ANG I). ANG I possesses almost no bioactivity and, upon action of angiotensin-converting enzyme (ACE), is converted to a bioactive form, ANG II. ANG II is a potent vasoconstrictor which plays a major role in increasing blood pressure. The vasoconstrictive effects of ANG II are produced by its action on the non-striated smooth muscle cells, the stimulation of the formation of the adrenergenic hormones epinephrine and norepinephrine as well as the increase of the activity of the sympathetic nervous system as a result of the formation of norepinephrine. In addition to this action, ANG II has proven to be active on the adrenal zona glomerulosa to induce aldosterone production and on the adrenal medulla and sympathetic nerve ends to promote catecholamine secretion, vasopressin secretion and prostaglandin E2 and I2 production, and is involved in the glomerular filtering function and the renal uriniferous tubular sodium reabsorption mechanism.

ANG II elicits its biological actions by binding to specific membrane bound receptors on target cells to activate multiple intracellular transduction pathways. ANG II acts at two major cellular receptors, angiotensin II type 1 receptor and angiotensin II type 2 receptor.

AT_1 receptor (AT_1R) and AT_2 receptor (AT_2R) belong to the class of G protein-coupled seven transmembrane receptors. AT_1R has been shown to mediate most of the traditionally recognized ANG II functions such as vasoconstriction, electrolyte homeostasis etc. There

has been evidence of generally antagonistic actions between the ANG II receptor isoforms AT_1R and AT_2R in the pressor and depressor actions and the growth promotion and suppression (Siragy H et al. 2001; Inagami et al. 1999). ANG II receptors are present in a number of organs and systems including heart, kidney, gonad, and placenta; pituitary and adrenal glands; the peripheral vessels, adipose tissue and the central nervous system. In kidney the major sites expressing AT_1R are glomeruli, proximal tubules, vasculature and medullary interstitial cells.

IGF-I has previously been shown to have vasodilatory effects and to improve cardiac function in healthy volunteers (Donath MY et al. 1998). IGF treatment has been associated with reduction of arteriolar resistance and an increase in capillary blood flow (Froesch E.R et al. 1994). Animal studies suggested a role for IGF-I as a mediator of cardiac hypertrophic responses (Delafontaine P. 1995). IGF-1 has been shown to have a beneficial effect on renal function in normal kidneys as well as those suffering from acute and chronic renal failure (Hirschberg R, et al. 1998).

The applicants have previously proven that an effective amount of IGF-I is successful in ameliorating or preventing hypertension which is a consequences of fetal programming ("Management of consequences of fetal programming" PCT/NZ01/00277, Vickers M.H, et al. 2001). Our research indicated that IGF-I treatment reduced systolic blood pressure (SBP) only in animals that were hypertensive as a result of fetal programming or postnatal hypercaloric nutrition, and systolic blood pressure in normotensive animals remained unaltered.

It has been shown that IGF-I can interact with the RAS and may alter ANG II expression via AT_1 receptor regulation. However, the studies of IGF-I effect on AT_1R carried out so far have focused on IGF-I activity in myocyte renin-angiotensin system and the inhibitory effect of IGF-I overexpression on apoptosis. It has been shown that in myocytes overexpressing IGF-I AT_1R protein was decreased further attenuating the response of myocytes to ANG II (Leri A, et al. 1999b). It has been suggested that the down-regulation of angiotensinogen (AGT), renin and AT_1R on myocytes and the reduced synthesis and secretion of AT_2 in the presence of IGF-I may be critical in the mechanism of prevention of cell death by IGF-I (Leri A, et al. 1999a) IGF-I was also found to interfere with the

development of diabetic myopathy by attenuating the activation of AT_1R (Kajstura J, et al. 2001).

None of the previous studies have shown the exact effect of IGF-I treatment on the density, distribution and signal transduction potential of ANG II receptors; the applicants' invention is the first of this kind to utilize IGF-I administration to modulate ANG II receptors in mammalian kidney. The applicants have previously speculated that the antihypertensive properties of IGF-I reported by them ("Management of the Consequences of Fetal Programming" PCT/NZ01/00277; Vickers et al. 2001) may potentially be attributed to IGF-I down-regulating the local RAS and limiting the formation of ANG II via mediation of the AT_1R. However, the precise effect which IGF-I compounds would exert on angiotensin II receptors was not elucidated by authors of the publication. Moreover, prior art literature taught that in vitro treatment of adrenal fasciculata-reticularis cells with IGF-I significantly increased AT_1R binding sites in those cells (Langlois et al. 1994).

The applicants' finding points to insulin-like growth factor-I (IGF-I) as a new alternative therapy or a co-therapy in a number of ANG II-mediated conditions, in particular hypertension. It has been proven in the literature that inhibition of the RAS by ACE inhibition or blockade of AT_1 receptors has a positive influence not only on hypertension but also brings about blood pressure (BP) independent renoprotective effects. (Adamczak et al. 2002). The selective ANG II receptor antagonism has been show to reduce insulin resistance and improve glucose tolerance. (Henriksen et al. 2001).

Administration of IGF-I not only achieves the benefits of selective ANG II receptor antagonism, like reduction of insulin resistance and improved glucose tolerance, but also has beneficial side effects not achieved by standard anti-hypertensive drugs. For example, blockade of age and fat mass regulated adipocyte angiotensin II receptors by IGF-I can prevent adipose tissue hypertrophy and ameliorates obesity.

Recent literature has shown that IGF-I receptors can function as G protein-coupled receptors (Dalle et al. 2001). Moreover, IGF-II receptors have been shown to interact with G proteins in a manner similar to that of conventional G receptor coupling, suggesting that a common G protein recognition mechanism is shared by IGF-II receptors and conventional G-coupled receptors (Nishimoto. 1993). ANG II receptors belong to the class of G protein-

coupled seven transmembrane receptors which are representative of a larger receptor family.

The present invention comprises the method of administration of IGF-I compounds to modulate the density, distribution and the potential for signal transduction of the G protein-coupled receptor family.

The novel application of IGF-I disclosed in the invention provides the public with a beneficial alternative to the methods of blocking or inhibiting the action of RAS existing in the prior art. Moreover, the present invention provides a new method of enhancing the efficacy of the present methods of inhibiting ANG II activity.

a. Definitions

In general, the following words or phrases or abbreviations have the indicated definition lwhen used in the description examples, and claims:

As used herein, "ANG II" means angiotensin II.

As used herein, "ANG II-like G protein-coupled seven transmembrane receptors" refer to any G protein-coupled seven transmembrane receptors characterised by pressor or depressor activity, similarly to angiontensin II receptors.

As used herein, "AT_1R" means angiotensin II type 1 receptor.

As used herein, "AT_2R" angiotensin II type 2 receptor.

As used herein, "angiotenisin II receptor" means a G protein-coupled seven transmembrane receptors which angiotensin II binds to and/or activates or which angiotensin II is capable of activating and/or binding to.

As used herein, "G protein-coupled seven transmembrane receptors" mean cell surface receptors that are coupled to G-proteins (GTP (guanosine 5'-triphosphate)-binding protein).

As used herein, "insulin-like growth factor" or "IGF-I" includes, IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs.

As used herein, IGF-I refers to any mammalian insulin-like growth factor-I IGF-I, examples being porcine IGF-I, ovine IGF-I, equine IGF-I, bovine IGF-I. It is, however, preferred that the IGF-I used be human IGF-I where the mammal is a human.

As used herein, the IGF-I is in native sequence or in variant form, and from any source, whether natural synthetic, or recombinant. Recombinant IGF-I can be obtained commercially.

As used herein, "a biologically active IGF-I analog" means a protein which is a variant of IGF-I through insertion, deletion or substitution of one or more amino acids, but which retains at least substantial functional equivalency. IGF-I and analogs can be purified from natural sources or produced by recombinant DNA techniques.

For the purposes of the present invention "a biologically active IGF-I analog" is also deemed to mean any compounds which exert a biological effect similar to IGF-I and which include but are not limited to any naturally occurring active parts of IGF-1 (e.g. GPE or des(1-3) IGF-I), IGF-2, any naturally occurring active parts of IGF-2 (e.g. des(1-3)IGF-II) or any of their known synthetic analogs. Synthetic analogs of IGF-I include, but are not limited to LR3IGF-I, [Arg^3]IGF-I, $Long^{TM}R^3$IGF-I, [Ala^{31}]IGF-I, Des(2,3)[Ala^{31}]IGF-I, [Leu^{24}]IGF-I, Des(2,3)[Leu^{24}]IGF-I, [Leu^{60}]IGF-I, [Ala^{31}][Leu^{60}]IGF-I, [Leu^{24}][Leu^{60}]IGF-I, etc.

The term "functionally equivalent ligand" means an agent which binds to and activates the receptors which IGF-I binds to and activates to give the required effect. A protein is a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has at least substantially the same function, as the original protein. The equivalent can be, for example, a fragment of the protein, a fusion of the protein with another protein or carrier, or a fusion of a fragment with additional amino acids. For example, it is possible to substitute amino acids in a sequence with equivalent amino acids using conventional techniques. Groups of amino acids normally held to be the equivalent are:

(a) Ala, Ser, Thr, Pro, Gly;

(b) Asn, Asp, Glu, Gln;

(c) His, Arg, Lys;

(d) Met, Leu, Ile, Val; and

(e) Phe, Tyr, Trp.

The present invention also extends to the administration of a compound which either increases the concentration of IGF-I, increases the concentration of analogs of IGF-I or

prevents inhibition of IGF-I activity. There is a wide variety of biological compounds that exert a stimulatory or inhibitory effect over IGF-1. Growth hormone, estrogen and thyroid hormone have all been reported to have stimulatory effects. There are also patents for design of IGF analogues (example US 6,251,865) that inhibit binding to IGF binding proteins, and for IGF inhibitors (example US 6,121,416) that prevent binding of IGF-1 to its receptor. Such options could also be used in the present invention.

As used herein "a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs, or a compound that prevents inhibition of IGF-I activity" also covers the acid-labile subunit (ALS) of the IGF-I binding complex.

AS used herein, "a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs, or a compound that prevents inhibition of IGF-I activity" also includes compounds which maintain, store, transport or prolong half-life of the IGF-I in circulation, in particular IGF-I binding proteins, for example those currently known, i.e., IGF-BP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, IGFBP7, IGFBP8.

As used herein, "density and distribution" refers to AT1 receptor immunolabelling within the mammalian kidney.

As used herein, "potential for signal transduction" or "signal transduction potential" refers to ability of the receptor to interact in the cascade of processes by which angiotensin II hormone peptide causes a change in the level of a second messenger for example calcium or cyclic AMP) and ultimately effects a change in the cells functioning.

As used herein, "hypertension" refers to persistently high arterial blood pressure. In humans this would normally equate to 140/90 systolic/diastolic ratio.

As used herein, "hypertension related kidney diseases" refers to any renal pathology related to hypertension.

As used herein, "ACE inhibitor" refers to drugs that exert haemodynamic effects mainly by inhibiting the RAS to produce a reduction of peripheral arterial resistance. These agents also modulate sympathetic nervous system activity and increase prostaglandin synthesis. They cause mainly vasodilation and mild natriuresis without affecting heart rate and contractility.

As used herein, "angiotensin II antagonist" refers to drugs that exert haemodynamic effects by blocking the binding of angiotensin II to the AT1 receptor.

b. Modes for carrying out the invention

In general, IGF-I compounds of this invention is directly administered to the mammal in therapeutically or prophylactically effective amounts by any suitable technique either singly, in combination with or in the presence of an ACE inhibitor or angiotensin antagonist.

IGF-I compound may be administered orally or parenterally, in combination with one or more suitable carriers or excipients. Preferably the IGF-1 is dissolved in sterile saline or water. The preferred administration route is subcutaneous injection. Another possibility is administration to the mammal of a replicable vehicle encoding the IGF-I/analogue/ligand. Such a vehicle (which may be a modified cell line or virus which expresses IGF-I/analogue/ligand within the mammal) has application in increasing the concentration of the active compound within the mammal for a prolonged period. Such a vehicle can form a part of an implant.

IGF-I dosage levels are formulation dependent due to volume load. The amount of IGF-1 that can be administered depends on the method of delivery. However, a suitable dosage range of IGF-I or IGF-I analogs formulated for injection is in the range of 0.1 μg/kg/day to 1 mg/kg/day. A preferred dosage rate is from about 2 to 200 μg/kg/day.

The invention will be more fully understood by reference to the following example. The example should not, however be construed as limiting the scope of the invention. The relevance of the findings to humans

All literature and patent citations are expressly incorporated by reference.

All animal work has been approved by the Animal Ethics Committee of the University of Auckland.

EXAMPLE 1

Materials and Methods

Virgin Wistar rats (age 100±5 days, n=15 per group) were time mated using a rat oestrous cycle monitor to assess the stage of oestrous of the animals prior to introducing the male.

After confirmation of mating, rats were housed individually in standard rat cages containing wood shavings as bedding and free access to water. All rats were kept in the same room with a constant temperature maintained at 25°C and a 12-h light:12-h darkness cycle. Animals were assigned to one of two nutritional groups: Group 1; undernutrition (30% of ad-libitum (UN)) of a standard diet throughout gestation, Group 2; standard diet (AD) throughout pregnancy. Food intake and maternal weights were recorded daily until birth. After birth, pups were weighed and litter size recorded. Pups from undernourished mothers were cross-fostered onto dams which received AD feeding throughout pregnancy. Litter size was adjusted to 8 pups per litter to assure adequate and standardised nutrition until weaning. After weaning, female offspring from the two groups of dams a) AD offspring and b) offspring from undernourished mothers (UN) were divided into 2 balanced postnatal nutritional groups to be fed either a standard diet (total digestible energy 2959kcal/kg, protein 19.4%, fat 5%, fat/energy ratio 15.21%, protein energy ratio 26.23) or a hypercaloric diet; (total digestible energy 4846kcal/kg, protein 31.8%, fat 30%, fat/energy ratio 55.72%, protein/energy ratio 26.25%). The mineral and vitamin content in the two diets were identical and in accordance with the requirements for standard rat diets. The fat content of the hypercaloric diet is typical of that seen in many Western diets. Weights and food intake of all offspring were measured daily for the first 2 weeks then every second day. At day 175, systolic blood pressure measurements were recorded using tail cuff plethysmography. Rats were then weight matched and received either rh-IGF-I (3μg/g/day) or saline by osmotic minipump (Model 2002, Alzet Corp, Palo Alto, Calif. US) for 14 days. On the day prior to sacrifice, a repeated systolic blood pressure was recorded. Rats were then fasted overnight and sacrificed by halothane anaesthesia followed by decapitation. Blood was collected into heparinised vacutainers and stored on ice until centrifugation and removal of supernatant for analysis All animal work was approved by the Animal Ethics Committee of the University of Auckland.

IGF-I infusion

At day 175, rats were weight matched (n = 6 per group) and received either rh-IGF-I (Genentech Code #G117AZ, Batch c9831AY) or saline by osmotic minipump (Model 2002, Alzet Corp, Palo Alto, Calif. US). The dose was 3μg/g/day for 14 days with a pump

delivery rate of 5µl per hour. The mean pump rate for the batch (Lot # 167258) of pumps used was 5.23 ± 0.2µl/hr. Pumps containing the IGF-I or saline solution were incubated in sterile saline for 4 hours at 37°C prior to implantation. The osmotic pumps were implanted subcutaneously, under halothane anaesthesia, using a small incision made in the skin between the scapulae. Using a haemostat, a small pocket was formed by spreading apart the subcutaneous connective tissues. The pump was inserted into the pocket with the flow moderator pointing away from the incision. The skin incision was then closed with sutures. All animals (n = 48) were housed individually for the duration of the study (WO 02/47714).

Tissue Sections

Kidney tissues were collected from offspring of undernourished Wistar rat mothers. Tissues were collected from 8 experimental groups (n= 6 per group), processes and then embedded in paraffin. Serial sections (5µm, 4 sections per animals, 2 sections per slide) were cut using a microtome (Leica, model RM2035), placed on poly-L-lysine coated slides and left to dry overnight in an incubator (Wilton utility incubator, UTIL72860, 57°C). After drying sections were deparaffinized with xylene and rehydrated with decreasing concentrations of alcohol through to PBS (0.01M) followed by distilled water. Tissues take from the following groups were examined:

UN control diet (UNC) saline *UN control diet (UNC) IGF-I treated*
UN high fat diet (UNHF) saline *UN high fat diet (UNC) IGF-I treated*

Immunohistochemistry for the AT_1R was performed using the avidin-biotin (ABC) method for immunostaining of paraffin embedded sections (Vectastain Elite ABC kit, Vector Laboratories, USA). In brief, 5µm sections were deparaffinized and treated with 1% H_2O_2 in methanol for 30 minutes at room temperature to inhibit endogenous peroxidase activity. Following this sections were washed in 0.01M PBS (pH 7.4) and incubated with 2.5% normal goat serum in 0.01M PBS (pH 7.4) containing 0.1% BSA (Lot 49284123, Roche). Sections were then incubated overnight at 4°C in a humidified chamber with a polyclonal anti-AT_1R antibody (SC-597, Santa Cruz Biotechnology, SC, USA) diluted in 0.1M PBS with 0.1% BSA (Lot 49284123, Roche). A series of antibody titres were investigated and

staining was optimised at a final primary antibody dilution ranging in the order of 1:50 to 1:100. After further washing, sections were incubated for 2 hours at room temperature with a biotinylated secondary antibody (Goat anti-rabbit IgG-Biotin). After a further washing step sections were incubated for 1 hour at room temperature with a avidin-biotin peroxidase complex (ABC). Immunoreactivity was then detected by the addition of diaminobenzidine (DAB) (Sigma, Lot 94H3677) and H_2O_2 in milli-Q water. Sections were then washed in 0.01M PBS (pH 7.4) and counterstained with Gills haematoxylin, dehydrated and mounted. Negative controls were performed by substituting the primary antibody with normal rabbit serum (Sigma, Lot 10H93113, G-0261) at a 1:200 dilution at 4°C overnight. This was done to identify any non-specific binding of the secondary antibody.

Evaluation of Sections

All sections were examined for differences in staining intensity and localisation of AT_1R staining with a light microscope at 400x magnification. Sections were analysed by an experienced observer blinded to the treatment groups to assess diet and treatment effects on receptor immunoreactivity. Sections were graded on a scale of 1 to 3, which ranged from low intensity (1), moderate intensity (2) through to high intensity staining (3).

Statistical analysis

Statistical analysis was carried out using the StatView statistical package (Version 5, SAS Institute, Cary, NC, USA). Differences in means between groups were determined by three-way (Glomerular Structure) and two-way (AT_1R immunoreactivity) ANOVA. Interaction effects between the various factors (diet, treatment and/or programming) were calculated and results were illustrated as histograms. Values were expressed as mean ± SEM. $p<0.05$ was taken as statistically significant.

Results

Immunohistochemistry for the AT_1R showed that postnatal hypercaloric nutrition did not affect the intensity and localisation of the AT_1R in the kidney of programmed animals. In contrast to this, all programmed animals that were treated with IGF-1 were observed to have a much lower intensity of staining of the AT_1R than their non-treated counterparts.

This is depicted in the photographs below (refer to Figures 1-10). Regions of brown staining reflect AT_1R immunoreactivity.

Effect	p-value
Diet	0.8672
Treatment	0.0284
Interactions:	
Diet x Treatment	0.3220

Table 3. Table of effects and p-value for the expression of the AT_1R in programmed kidneys
$p < 0.05$ is considered statistically significant.

There were no significant differences in the expression of the AT_1R as a consequence of postnatal hypercaloric nutrition. However IGF-1 treatment in programmed animals decreased the staining intensity and localisation of the AT_1R ($p<0.05$).
There is notable difference between the expression of the AT_1R protein in the kidney of programmed animals and their IGF-1 treated counterpart. There appears to be a link between the decreased expression of the AT_1R in IGF-1 treated programmed offspring and the finding that IGF-1 decreases blood pressure in programmed offspring.

Summary

IGF-1 treatment was shown to significantly ($p<0.05$) reduce the expression of the AT_1R protein in the kidneys of programmed offspring. This IGF-1 mediated AT_1R expression, suggests a possible mechanism by which angiotensin II formation can be reduced, consequently reducing systolic pressure.

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What is claimed is:

1. A method for modulating the density, distribution and/or the potential for signal transduction of angiotensin II receptors or angiotensin II-like G protein-coupled seven transmembrane receptors in a mammalian tissue comprising the step of administering an effective amount of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs to a mammal.

2. The method as claimed in claim 1 wherein the angiotensin II receptors are angiotensin II type 1 receptors and wherein their density, distribution, and potential for signal transduction are down-regulated.

3. The method as claimed in claim 1 wherein the angiotensin II receptors are angiotensin II type 2 receptors and wherein their density, distribution and potential for signal transduction are up-regulated.

4. The method as claimed in claim 1 wherein the mammal is human.

5. The method as claimed in claim 1 wherein the mammalian tissue is renal tissue.

6. The method as claimed in claim 1 wherein the effective amount of an insulin-like growth factor-I (IGF-I) compound is administered in a form of a pharmaceutical composition including a pharmaceutically acceptable carrier thereof.

7. The method as claimed in claim 1 wherein the effective amount of IGF-I compound is administered by way of administration of a replicable vehicle encoding for said IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs.

8. The method as claimed in claim 1 wherein the effective amount of IGF-I compound is administered by intramuscular, subcutaneous or intraperintoneal injection or implant.

9. The method as claimed in claim 1 wherein the said effective amount of IGF-I compound is administered through intravenous, transdermal, transmucosal, oral, or epidural route.

10. The method as claimed in claim 1 wherein the effective amount of an insulin-like growth factor-I (IGF-I) compound is between 0.1 μg/kg/day and about 1 mg/kg/day.

11. The method as claimed in claim 1 wherein the increase of the concentration of IGF-I or IGF-I analog is from about 0.1 μg/kg/day to about 1mg/kg/day.

12. A method for modulating the density, distribution and/or the potential for signal transduction of G protein-coupledseven transmembrane receptors in a mammalian tissue comprising the step of administering an effective amount of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs to a mammal.

13. A use of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs in the preparation of a medicament for modulating the density and the potential for signal transduction of angiotensin II receptors in a mammalian tissue.

14. The use as claimed in claim 13 wherein the medicament is administered in a pharmaceutically acceptable combination with one or more suitable carriers or excipients.

15. The use as claimed in claim 13 wherein the medicament is used for treatment, prophylaxis, attenuation of hypertension in the mammal.

16. The use as claimed in claim 13 wherein the medicament is used for treatment, prophylaxis, attenuation of resulting from hypertension related kidney diseases in a mammal.

17. The use as claimed in claim 13 wherein the medicament is administered in the presence of ACE inhibitors or angiotensin II antagonists.

18. The use of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs for the manufacture of a medicament for modulation of the density, distribution or potential for signal transduction of G protein-coupled seven transmembrane receptors in a mammalian tissue.

19. A method for enhancing the antihypertensive and renoprotective properties of ACE inhibitors and angiotensin II antagonists comprising the step of administering to a mammal an effective amount of an insulin-like growth factor-I (IGF-I) compound, where an IGF-I compound comprises IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs in combination with the said ACE inhibitor or the said angiotensin II antagonist.

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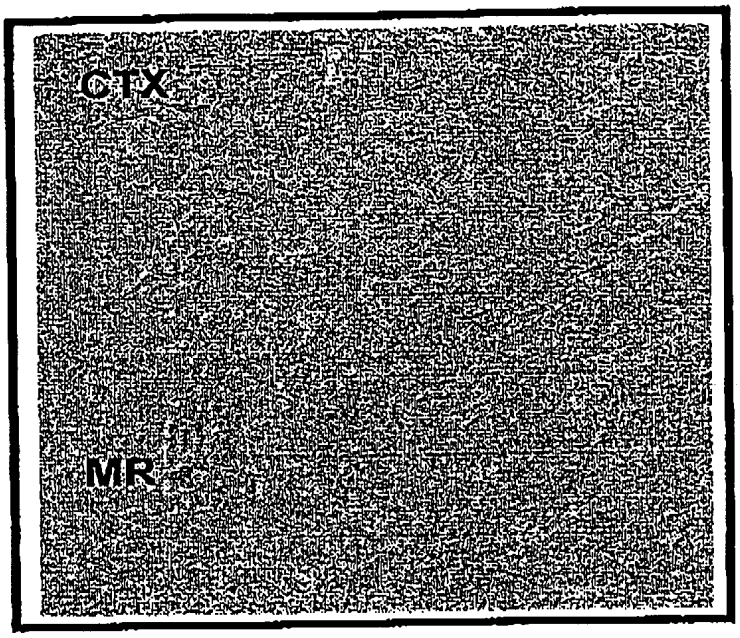

FIGURE 1

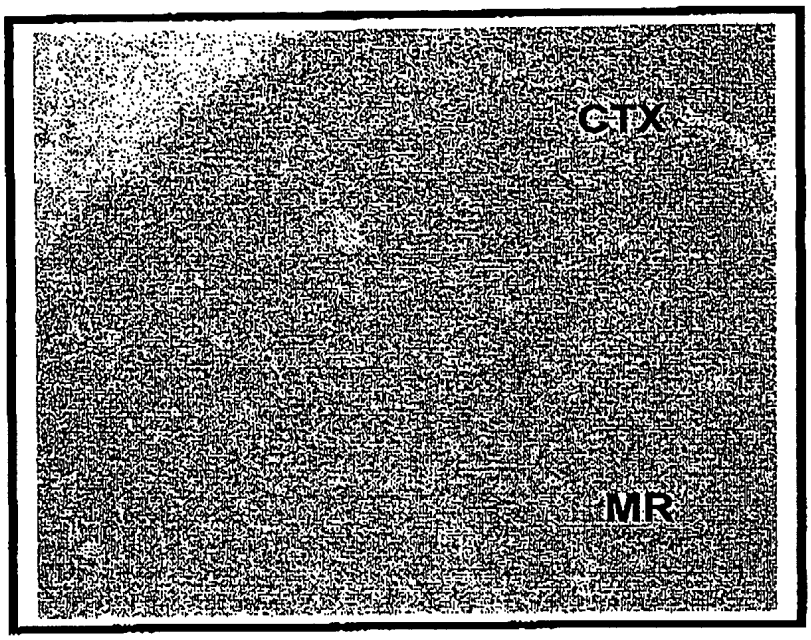

FIGURE 2

FIGURE 3

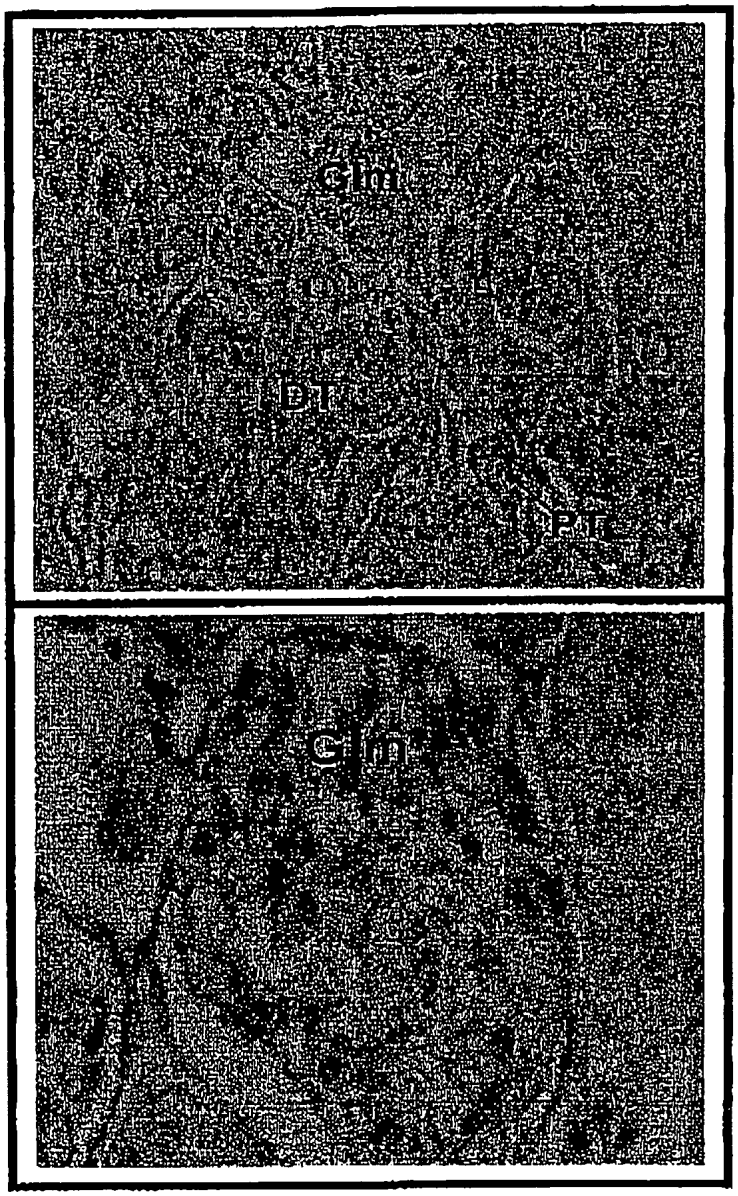

FIGURE 4

FIGURE 5

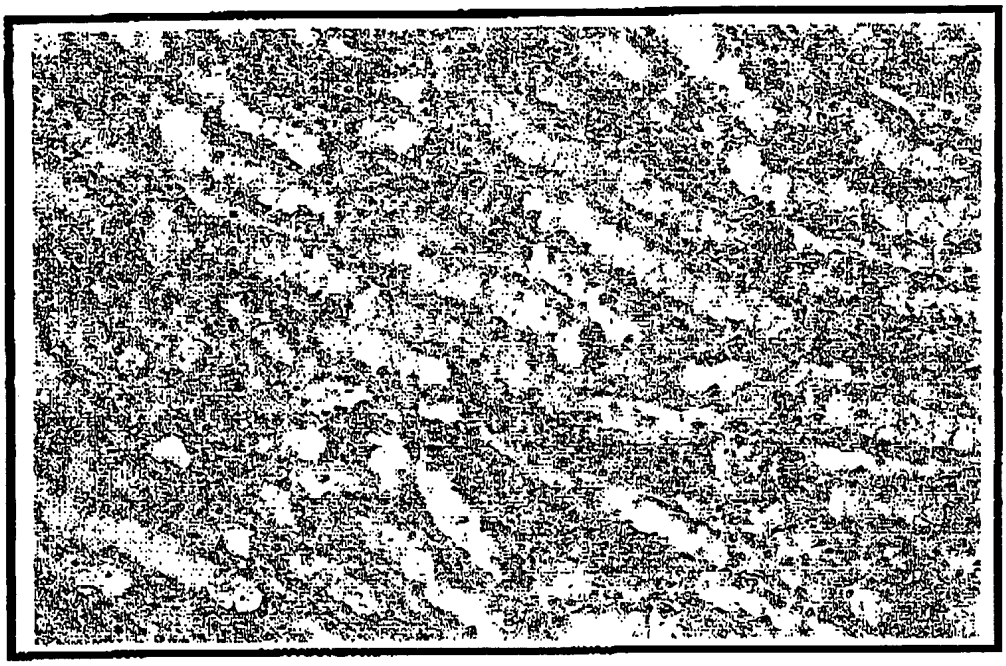

FIGURE 6

FIGURE 7

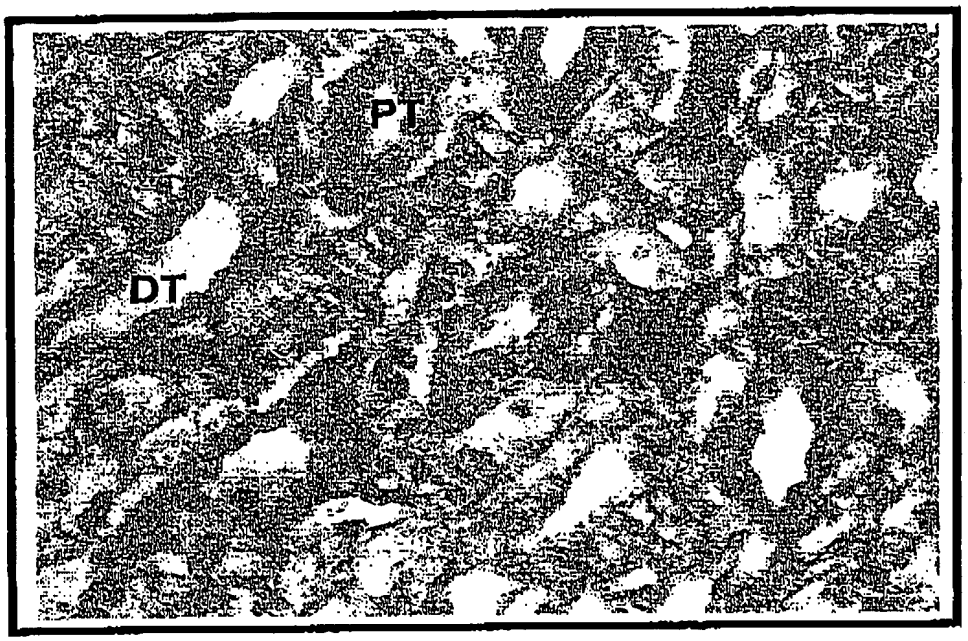

FIGURE 8

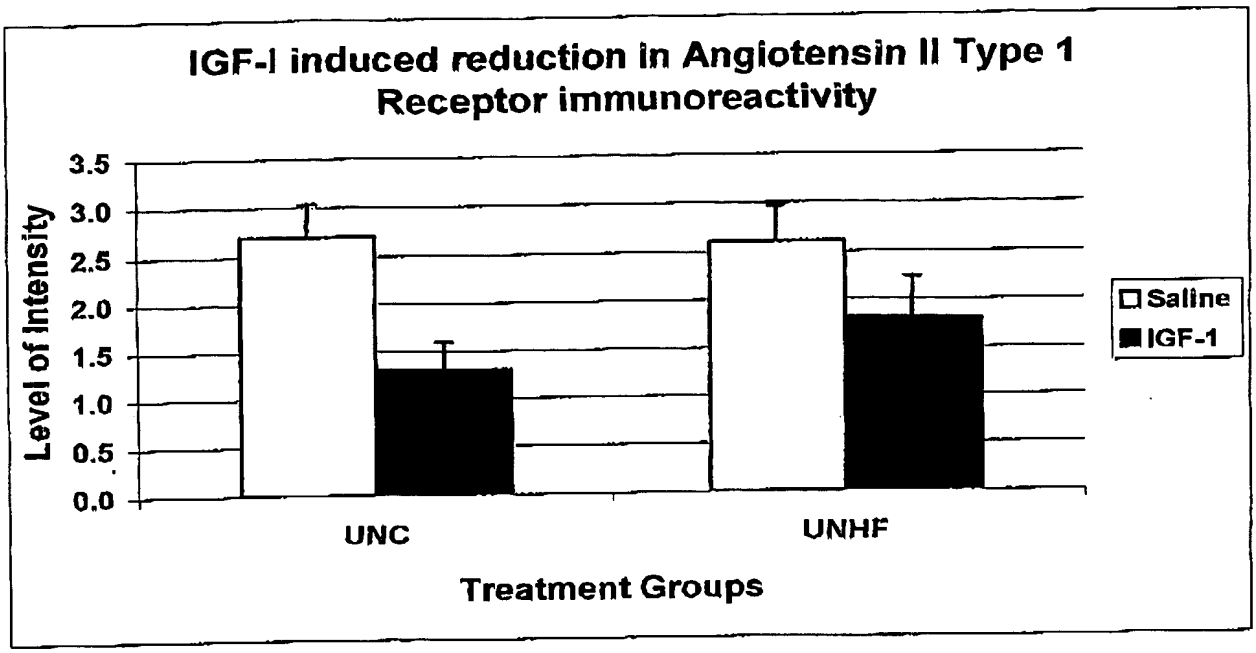
IGF-I induced reduction in Angiotensin II Type 1 Receptor immunoreactivity
Level of Intensity
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
UNC
UNHF
Treatment Groups
Saline
IGF-1

FIGURE 9

ABSTRACT

A therapeutic and prophylactic applications of insulin-like growth factor-I (IGF-I) compound are disclosed comprising administering IGF-I, a biologically active IGF-I analog, a biologically active IGF-I mimetic, a functionally equivalent ligand, a compound that increases the concentration of IGF-I, or a compound that increases the concentration of IGF-I analogs to a mammal to modulate the density, distribution and the potential for signal transduction of angiotensin II and angiotensin II-like G protein-coupled seven transmembrane receptors in mammalian renal tissue.

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